

## ANTI-ANAPHYLACTIC ACTIVITY OF *HELIOTROPIUM INDICUM* LEAVES

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### ABSTRACT

The alcoholic extract of *Heliotropium indicum* leaves was evaluated using Wistar rats and Duncan Hartley guinea pigs. The anti-anaphylactic activity was investigated in rats using the active anaphylaxis model. The effect on mast cell stabilization was performed by *ex vivo* challenge of antigen in sensitized rat intestinal mesenteries. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where preconvulsive dyspnea was used as an end point following exposure to histamine aerosol. Treatment with *Heliotropium indicum* at 500 mg/kg, p.o. showed significant reduction in signs and severity of symptoms ( $P < 0.05$ ), onset ( $P < 0.001$ ) and mortality rate ( $P < 0.05$ ) following anaphylactic shock-induced bronchospasm. Treatment of sensitized animals with *Heliotropium indicum* at 500 mg/kg, p.o. for 2 weeks resulted in a significant reduction in the number of disrupted mast cells ( $P < 0.001$ ) when challenged with an antigen (horse serum). *Heliotropium indicum* significantly prolonged the latent period of convulsion ( $P < 0.008$ ) as compared to control following exposure of guinea pigs to histamine aerosol. The findings from various studies reveal that the antihistaminic and anti-anaphylactic activity of *Heliotropium indicum* may be due to the mast cell stabilizing potential, suppression of IgE, and inhibition of release of inflammatory mediators.

**KEYWORDS:** anaphylactic, histaminic, Hypersensitivity, IgE, mast cell degranulation

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### INTRODUCTION

Life, disease and decay are inseparable. From his first awakening, man has sought to fight and control diseases. He turned to nature for inspiration and guidance. Herbs have been used as a source of drugs to combat diseases since time immemorial. The effectiveness, easy availability, low cost and non-toxic nature popularized herbal remedies.<sup>1</sup>

*Heliotropium indicum* (Boraginaceae) - commonly called as Indian Turnsole, is a herb with slightly woody at base. It is distributed in the tropical and temperate regions of the world and found throughout India. The whole plant is claimed to possess medicinal properties. In ayurveda the juice of leaves applied on boils, pimples, ulcers, sores and wounds to cure. The plant used for diarrhea, malaise or vomiting in infants. The leaves are used for the treatment of ophthalmic and allergic disorders, erysipelas, pharyngodynia, anti-tumor and anti-inflammatory. The roots are used as astringent, expectorant and febrifuge. The extract of leaves was proved to be active against Schwart's leukemia.<sup>2</sup>

However there is no scientific data available to authenticate the folklore claim. Hence the present study was undertaken to evaluate the anti-anaphylactic activity of ethanolic extract of *Heliotropium indicum* leaves.

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Allergic diseases are responsible for significant morbidity and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the disorders.<sup>3</sup> Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulins, mast cells, and various autacoids in the etiopathogenesis of allergic conditions. In spite of the voluminous

literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events, and compliance issues.<sup>4</sup>

In the present study, the effect of alcoholic extract of *Heliotropium indicum* was studied on the active anaphylaxis and mast cell stabilization in rats and histamine-induced bronchospasm in guinea pigs.

## **MATERIALS AND METHODS**

### **Plant material**

The leaves of *H.indicum* were collected from Udupi, Karnataka. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India.

### **Preparation of Extract**

Leaves were shade dried and powdered mechanically. The powder was loaded into Soxhlet extractor (250 g) each and was subjected to extraction for about 36–40 h with 95% ethanol. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using a rotary flash evaporator to a syrupy consistency. Then it was dried in the dessicator. The yield was about 12% w/w.

### **Animals**

Wistar rats (175-200 g) and guinea pigs (400-600 g) of either sex are procured from inbred animal house facility of Srinivas College of Pharmacy, Mangalore. They are maintained under standard conditions (temperature  $22 \pm 2C^0$ , relative humidity  $60 \pm 5\%$  and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

### **Drugs and Chemicals**

Histamine and horse serum were procured from Sigma Chemicals and toluidine blue from Loba-Chemie, Mumbai. All other chemicals and reagents were procured from Hi-Media Laboratories limited, Mumbai.

### **Active anaphylaxis**

Twenty eight rats were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml of triple antigen containing 20,000 million *Bordetella pertussis* organisms. The sensitized rats were divided into 4 groups of 7 each. Group I served as control and

received water (vehicle). Groups II, III and IV were administered *Heliotropium indicum* at 125, 250 and 500 mg/kg respectively, orally, once a day for 14 days. On day 14, after 2 h of treatment, the rats were challenged with intravenous injection (tail vein) of 0.25 ml horse serum in normal saline. They were then observed for the onset of symptoms such as dyspnea and cyanosis, duration of the persistence of symptoms (min), and mortality. The severity of symptoms was scored.<sup>5</sup>

### **Mast cell stabilizing activity**

Thirty-two rats were divided into four groups of eight animals in each group. Group I served as control and received vehicle (water). Group II (sensitized control group, received only water), Groups III and IV were sensitized by injecting 0.5 ml of horse serum subcutaneously along with 0.5 ml of triple antigen containing 20,000 million *Bordetella pertussis* organisms. Group III were administered *Heliotropium indicum* 500 mg/kg, p.o., once a day for 14 days. Group IV were administered Prednisolone (reference drug) 10 mg/kg, p.o., for the same duration. On day 14, the rats were sacrificed 2 h after the treatment and the intestinal mesentery was taken out for the study on mast cells. Mesenteries along with intestinal pieces were excised and kept in Ringer Locke solution (NaCl 154, KCl 5.6, CaCl<sub>2</sub> 2.2, NaHCO<sub>3</sub> 6.0, glucose 5.55 mM/L of distilled water) at 37oC. The mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained with 1.0% toluidine blue and examined microscopically for the number of intact and degranulated mast cells.<sup>5</sup>

### **Histamine-induced bronchospasm in guinea pigs**

Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure (1 kg/cm<sup>2</sup>) in an aerosol chamber (24 × 14 × 24 cm) made of perplex glass. Of the two groups of six animals each, Group I served as control and Group II received *Heliotropium indicum* 500 mg/kg, p.o., once a day for 5 days. The animals were exposed to 1% histamine aerosol under constant pressure (1 kg/cm<sup>2</sup>) in an aerosol chamber on day 0 without any treatment. The end point, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from the chamber and exposed to fresh air. This PCD was taken as day 0 value. On days 1 and 5, 2 h after the administration of the drug, the time for the onset of PCD was recorded as on day 0.<sup>6</sup>

### **Statistical analysis**

The results of various studies were expressed as mean  $\pm$  SEM and analyzed statistically using one-way ANOVA,

followed by Bonferroni's multiple comparison post-hoc test or chi-square test or unpaired Student's 't' test to find out the level of significance.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Effect of *Heliotropium indicum* on anaphylactic shock-induced bronchospasm in sensitized rats

*Heliotropium indicum* protected the sensitized rats against anaphylactic shock in a dose-dependent manner. In control rats, intravenous antigen challenge (horse serum) caused shock in 100% of the animals, while in treated rats (500 mg/kg of *Heliotropium indicum*), the onset of symptoms of shock was delayed ( $P < 0.001$ ), and symptoms were less severe ( $P < 0.05$ ) with reduced mortality ( $P < 0.05$ ). *Heliotropium indicum* showed optimal pharmacological effect at 500 mg/kg. Hence, this dose chosen for the remaining studies (Table 1).

### Mast cell stabilizing potential of *Heliotropium indicum*

Antigen challenge resulted in significant degranulation of the mesenteric mast cells (approximately 88%,  $P < 0.001$ ). Pretreatment of sensitized animals with *Heliotropium indicum* at 500 mg/kg, p.o., for 2 weeks resulted in a significant reduction in the number of disrupted mast cells ( $P < 0.001$ ) when challenged with horse serum. The effect of *Heliotropium indicum* was also comparable with the reference drug prednisolone (Table 2).

### Effect of *Heliotropium indicum* on histamine-induced bronchospasm

*Heliotropium indicum* at 500 mg/kg, p.o., significantly prolonged the latent period of PCD ( $P < 0.008$ ) as compared to control, following exposure to histamine aerosols on day 5 (Table 3).

## DISCUSSION

Experimental animal model of asthma is characterized by allergen-induced immediate airway constriction and late airway reactivity to a pharmacological vasoconstrictor such as histamine and leukotrienes. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders. In the present study, *Heliotropium indicum* prolonged the latent period of PCD in guinea pigs following histamine aerosol. This may be suggestive of an antihistaminic activity following treatment with *Heliotropium indicum*. It also offered protection against anaphylactic shock-induced bronchospasm in rats.

Basophils, mast cells, and their preformed *de novo* synthesized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they

modulate local immune responses and inflammatory cell infiltration.<sup>7, 8</sup> Immunoglobulin E (IgE)-mediated mast-cell stimulation is an important initial event in the development of type I allergic reactions such as asthma and atopic disorders. Clinical studies have found a close association between asthma and serum IgE levels, as well as IgE-dependent skin test reactivity to allergens.<sup>9</sup> Antigen challenge, in sensitized animals, results in the degranulation of mast cells, which is an important feature of anaphylaxis. In the present study, *Heliotropium indicum* showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of *Heliotropium indicum* may be attributed to the presence of herbal extracts, which are known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation mast cells.<sup>9</sup> This antianaphylactic and antihistaminic effect may be caused by the stabilization of the mast cell membrane, suppression of IgE, and inhibition of pathological effects induced by the release of inflammatory mediators in *Heliotropium indicum* treated animals.

All the above findings lend credence to the beneficial use of *Heliotropium indicum* in the treatment of asthma and related conditions. However, further studies with other experimental models, especially to explore the role of cytokines are warranted to substantiate the antiasthmatic and antiallergic activity of *Heliotropium indicum*.

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TABLE 1. EFFECT OF H.*INDICUM* ON ANAPHYLACTIC SHOCK INDUCED BRONCHOSPASM

Groups	Scores/min/percentage		
	Total score x 10	Onset of symptoms (min)	Mortality
Control	123	72	100
H <i>Indicum</i> (125mg/kg)	116	68	96
H <i>Indicum</i> (250mg/kg)	89	84	78
H <i>Indicum</i> (500mg/kg)	66*	108#	52*

Values are mean ± SEM except for mortality, which is expressed as percentage, n=7 in each group; Total score: F=5.508, df=27, P=0.005; Onset of symptoms: F=20.51, df = 27, P=0.0001. \*P<0.05, #P<0.001 as compared to control. (ANOVA followed by Bonferroni's multiple comparison post hoc tests for total score and onset of symptoms. Chi-square test for mortality).

TABLE 2. EFFECT OF H.*INDICUM* ON MAST CELL STABILIZATION IN SENSITIZED RATS

Groups	Mast cells (%)	
	Intact	Disrupted
Control	81.06±3.70*	18.94±3.70*
Sensitized control	12.30±1.87	87.70±1.87
H <i>Indicum</i> (500mg/kg)	60.12±7.53*	39.88±7.53*
Prednisolone (10mg/kg)	70.11±3.89*	29.89±3.89*
One-Way F	123.8	123.8
ANOVA	30	30
P	0.001	0.001

Values are mean ± SEM, n=8 in each group. \* Significantly different from sensitized control (P<0.001), Bonferroni's multiple comparison post hoc test

TABLE 3. EFFECT OF H.*INDICUM* ON HISTAMINE-INDUCED BRONCHOSPASM IN GUINEA PIGS

Groups	Pre-convulsive dyspnea (sec)		
	Day 0	Day 1	Day 5
Control	190	192	190
H <i>Indicum</i> (500mg/kg)	188	197	320*
P	0.008	0.008	0.008

Values are mean ± SEM, n=6 in each group. \* (P<0.008) as compared to control on day 5 (Unpaired Student's 't' test).

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